CLAIMS LISTING

1. (Amended) A method for screening a molecule, wherein said molecule is a chemical compound, or a drug which ~~have~~ has a synthetic lethal property, when in combination with a gene of interest carrying a non-lethal mutation, said method comprising the steps of:

i. transfecting a first reporter gene, as part of an integration plasmid, into mammalian cells having a genome comprising a gene of interest which carries a non-lethal mutation, or a genome which is null of said gene of interest;

ii. selecting clones stably expressing said first reporter gene;

iii. introducing into said cells a survival plasmid comprising a functioning copy of said gene of interest, a second reporter gene, selectable marker, an origin of DNA replication and a nuclear antigen gene essential for replication of the plasmid within said cells, wherein said survival plasmid is autonomously replicating and spontaneously lost from said cells;

vi. growing said cells in the presence of a selection compound which selects for said selectable marker;

vii. selecting cell clones stably expressing said second reporter gene and said functioning copy of said gene of interest;

viii. removing said selection compound, ~~for the~~ which selects for said selectable marker, and adding molecules destined for screening of their ability to impose selective pressure enforcing retention of the unstable survival plasmid.

ix. determining survival plasmid retention in cells by measuring the expression ratio of second's to first reporter gene, wherein, if the survival plasmid retains, the molecule has ~~thus identifying a molecule having~~ a synthetic lethal property when in combination with a non lethal mutated gene of interest.

2. (Original) The method according to Claim 1, wherein said selectable marker is a dominant selectable marker.

3. (Original) The method according to Claim 1, wherein said cells are human cells.

4. (Original) The method according to Claim 1, wherein said cells are rodent cells.

5. (Amended) The method according to Claim 1, wherein the products of said first reporter gene and second reporter gene are fluorescent proteins.

6. (Original) The method according to Claim 5, wherein the product of said first reporter gene has an excitation and/or emission peak which differs from the excitation and/ or emission peak of the product of said second reporter gene.

7. (Amended) The method according to Claim ~~1~~ 3, wherein said human cells are human cancer cells.

8. (Original) The method according to Claim 7, wherein said gene of interest is specifically incapacitated in human cancer cells.

9. (Cancelled) ~~The method of claim 1, wherein said molecule is a chemical compound, an antisensedeoxyoligonucleotide, , ribozymes, RNA aptamers, a synthetic small interfering RNA (siRNA), and peptide aptamers.~~

10-12 (were omitted in the original)

13. (Withdrawn) A method for screening a cDNA molecule, which have a synthetic lethal property when in combination with a gene of interest carrying a non-lethal mutation, said method comprising the steps of:

i. transfecting a first reporter gene into a mammalian cells having a genome comprising a gene of interest which carries a non-lethal mutation;

ii. selecting clones stably expressing said first reporter gene;

iii. introducing into said cells a survival plasmid comprising a functioning copy of said gene of interest, a second reporter gene, a selectable marker, an origin of DNA replication and a nuclear antigen gene essential for replication of the plasmid within said cells, wherein said plasmid is spontaneously lost from said cells;

iv. growing said cells in the presence of a selection compound which selects for said selectable marker;

v. selecting cell clones stably expressing said second reporter gene and said functioning copy of said gene of interest;

vi. incorporating said cDNA molecule- into a vector vehicle containing a second selectable marker gene so as to obtain a vector vehicle-cDNA molecule.

vii. transfecting cells with vector vehicles-cDNAs molecules while removing selection for the first selectable marker, and instituting selection for pools of cells expressing the second selectable marker gene.

viii. determining survival plasmid retention in cells, thus identifying a cDNA having a synthetic lethal property when in combination with a non lethal mutated gene of interest

14. (Withdrawn) The method according to Claim 13, wherein said cDNA is full-length or partial-length/truncated cDNA, or cDNA of full or truncated length in antisense orientation.

15. (Withdrawn) The method of claim 13, wherein said vector vehicle is episomal mammalian expression vector, a retroviral vector, aDNA- or RNA-based autonomously replicating viral vector, and a chimeric transposable element.

16-18 (were omitted from the original)

18. (Withdrawn) The method according to Claim 13, wherein said selectable marker is a dominant selectable marker.

19. (Withdrawn) The method according to Claim 13, wherein said cells are human cells.

20. (Withdrawn) The method according to Claim 13, wherein said cells are rodent cells.

21. (Withdrawn) The method according to Claim 13, wherein the products of said first and second reporter genes are fluorescent proteins.

22. (Withdrawn) The method according to Claim 21, wherein the product of said first reporter
gene has an excitation and/ or emmission peak which differs from the excitation and/ or emmission peak of the product of said second reporter gene.

23. (Withdrawn) The method according to Claim 13, wherein said human cells are human cancer cells.

24. (Withdrawn) The method according to Claim 13, wherein said gene of interest is specifically incapacitated in human cancer cells.

25. (Withdrawn) The method of claim 13, wherein step viii further comprises the step of FACS sorting leading to enrichment or isolation of cells retaining the survival plasmid.

28. (Withdrawn) A method for screening a drug which have a synthetic lethal property when in combination with a gene of interest carrying a non-lethal mutation, said method comprising the steps of:

i. transfecting a first reporter gene into a non-yeast eukaryotic cells having a genome comprising a gene of interest which carries a non-lethal mutation;

ii. selecting clones stably expressing said first reporter gene;

iii. introducing into said cells a survival plasmid comprising a functioning copy of said gene of interest, a second reporter gene, a selectable marker, an origin of DNA replication and a nuclear antigen gene essential for replication of the plasmid within said cells, wherein said survival plasmid is spontaneously lost from said cells;

iv. growing said cells in the presence of a selection compound which selects for said selectable marker;

v. selecting cell clones stably expressing said second reporter gene and said functioning copy of said gene of interest;

vi. adding the drugs destined for screening their ability to impose selective pressure enforcing retention of the spontaneously lost survival plasmid

vii. determining survival plasmid retention in cells, thus identifying a drug having a a synthetic lethal property when in combination with non lethal mutated gene of interest.

29. (Withdrawn) The method according to Claim 28, wherein said selectable marker is a dominant selectable marker.

30. (Withdrawn) The method according to Claim 28, wherein said cells are human cells.

31. (Withdrawn) The method according to Claim 28, wherein said cells are rodent cells.

32. (Withdrawn) The method according to Claim 28, wherein the products of said first and second reporter genes are fluorescent proteins.

33. (Withdrawn) The method according to Claim 32, wherein the product of said first reporter gene has an excitation and/ or emmission peak which differs from the excitation and/ or emmission peak of the product of said second reporter gene.

34. (Withdrawn) The method according to Claim 28, wherein said human cells are human cancer cells.

35. (Withdrawn) The method according to Claim 34, wherein said gene of interest is specifically incapacitated in said human cancer cells.

36. (Withdrawn) The method of claim 28, wherein said drug is a chemical compound, an antisensedeoxyoligonucleotide, , ribozymes, RNA aptamers, synthetic small interfering RNA (siRNA) and peptide aptamers.

37. (Withdrawn) A method for screening a library comprising a plurality of molecules which have a synthetic lethal property when in combination with a gene of interest carrying a non-lethal mutation, said method comprising the steps of:

i. transfecting a first reporter gene into mammalian cells having a genome comprising a gene of interest which carries a non-lethal mutation;

ii. selecting clones stably expressing said first reporter gene;

iii. introducing into said cells a survival plasmid comprising a functioning copy of said gene of interest, a second reporter gene, a selectable marker, an origin of DNA replication and a nuclear antigen gene essential for replication of the plasmid within said cells, wherein said plasmid is spontaneously lost from said cells;

vi. growing said cells in the presence of a selection compound which selects for said selectable marker;

v. selecting cell clones stably expressing said second reporter gene and said functioning copy of said gene of interest;

vi. adding the library comprising a plurality of molecules in order to identify those that impose selective pressure enforcing the retention of the spontaneously lost survival plasmid

vii. determining survival plasmid retention in cells, thus identifying at least onemolecule within a library having a synthetic lethal property when in combination with a non lethal mutated gene of interest.

38. (Withdrawn) The method according to Claim 37, wherein said selectable marker is a dominant selectable marker.

39. (Withdrawn) The method according to Claim 38, wherein said cells are human cells.

40. (Withdrawn) The method according to Claim 38, wherein said cells are rodent cells.

41. (Withdrawn) The method according to Claim 38, wherein the products of said first and second reporter genes are fluorescent proteins.

42. (Withdrawn) The method according to Claim 41, wherein the product of said first reporter gene has an excitation and/ or emmission peak which differs from the excitation and/ or emmission peak of the product of said second reporter gene.

43. (Withdrawn) The method according to Claim 37, wherein said human cells are human cancer cells.

44. (Withdrawn) The method according to Claim 43, wherein said gene of interest is specifically incapacitated in said human cancer cells.

45. (Withdrawn) The method of claim 41, wherein step vii further comprises the step of FACS sorting in order to enrich or isolat cells which retain the survival plasmid.

46. (Withdrawn) A method for screening molecule which have a synthetic lethal property when in combination with a mutant or normal gene of interest which is overexpressed, said method comprising the steps of:

i. transfecting a first reporter gene into mammalian cells having a genome comprising a mutant or normal gene of interest which is overexpressed,

ii. selecting clones stably expressing said first reporter gene;

iii. introducing into said cells a survival plasmid comprising a dominant-negative mutant of said gene of interest, a second reporter gene, selectable marker, an origin of DNA replication and a nuclear antigen gene essential for replication of the plasmid within said cells, wherein said survivsal plasmid is autonomously replicating and spontaneously lost from said cells;

vi. growing said cells in the presence of a selection compound which selects for said selectable marker;

vii. selecting cell clones stably expressing said second reporter gene and said dominant-negative mutant of said gene of interest;

viii. removing selection for the selectable marker, and adding molecules destined for screening of their ability to impose selective pressure enforcing retention of the unstable survival plasmid.

ix. determining survival plasmid retention in cells, thus identifying a molecule having a synthetic lethal property when in combination with the a mutant or normal gene of interest which is overexpressed.

47. (Withdrawn) The method according to Claim 46, wherein said selectable marker is a dominant selectable marker.

48. (Withdrawn) The method according to Claim 46, wherein said cells are human cells.

49. (Withdrawn) The method according to Claim 46, wherein said cells are rodent cells.

50. (Withdrawn) The method according to Claim 46, wherein the products of said first and second reporter genes are fluorescent proteins.

51. (Withdrawn) The method according to Claim 50, wherein the product of said first reporter gene has an excitation and/ or emmission peak which differs from the excitation and/ or emmission peak of the product of said second reporter gene.

52. (Withdrawn) The method according to Claim 46, wherein said human cells are human cancer cells.

53. (Withdrawn) The method according to Claim 52, wherein said gene of interest is specifically incapacitated in human cancer cells.

54. (Withdrawn) The method of claim 46, wherein said molecule is a chemical compound, an antisensedeoxyoligonucleotide, , ribozymes, RNA aptamers, a synthetic small interfering RNA (siRNA), and peptide aptamers.

55. (Withdrawn) A method for screening a cDNA molecule, which have a synthetic lethal property when in combination with a mutant or normal gene of interest which is overexpressed, said method comprising the steps of:

i. transfecting a first reporter gene into a mammalian cells having a genome comprising a mutant or normal gene of interest which is overexpressed;

ii. selecting clones stably expressing said first reporter gene;

iii. introducing into said cells a survival plasmid comprising a dominant-negative mutant of said gene of interest, a second reporter gene, a selectable marker, an origin of DNA replication and a nuclear antigen gene essential for replication of the plasmid within said cells, wherein said plasmid is spontaneously lost from said cells;

iv. growing said cells in the presence of a selection compound which selects for said selectable marker;

v. selecting cell clones stably expressing said second reporter gene and said dominant-negative mutant of said gene of interest;

vi. incorporating said cDNA molecule- into a vector vehicle containing a second selectable marker gene so as to obtain a vector vehicle-cDNA molecule.

vii. transfecting cells with vector vehicles-cDNAs molecules while removing selection for the first selectable marker, and instituting selection for pools of cells expressing the second selectable marker gene.

viii. determining survival plasmid retention in cells, thus identifying a cDNA having a synthetic lethal property when in combination with the a mutant or normal gene of interest which is overexpressed.

56. (Withdrawn) The method according to Claim 55, wherein said cDNA is full-length or partial-length/truncated cDNA, or cDNA of full or truncated length in antisense orientation.

57. (Withdrawn) The method of claim 55, wherein said vector vehicle is episomal mammalian expression vector, a retroviral vector, a DNA- or RNA-based autonomously replicating viral vector, and a chimeric transposable element.

58. (Withdrawn) The method according to Claim 55, wherein said selectable marker is a dominant selectable marker.

59. (Withdrawn) The method according to Claim 55, wherein said cells are human cells.

60. (Withdrawn) The method according to Claim 55, wherein said cells are rodent cells.

61. (Withdrawn) The method according to Claim 55, wherein the products of said first and second reporter genes are fluorescent proteins.

62. (Withdrawn) The method according to Claim 61, wherein the product of said first reporter gene has an excitation and/ or emmission peak which differs from the excitation and/ or emmission peak of the product of said second reporter gene.

63. (Withdrawn) The method according to Claim 55, wherein said human cells are human cancer cells.

64. (Withdrawn) The method according to Claim 55, wherein said gene of interest is specifically incapacitated in human cancer cells.

65. (Withdrawn) The method of claim 55, wherein step viii further comprises the step of FACS sorting leading to enrichment or isolation of cells retaining the survival plasmid.

66. (Withdrawn) A method for screening a drug which have a synthetic lethal property when in combination with a mutant or normal gene of interest which is overexpressed , said method comprising the steps of:

i. transfecting a first reporter gene into a non-yeast eukaryotic cells having a genome comprising a mutant or normal gene of interest which is overexpressed;

ii. selecting clones stably expressing said first reporter gene;

iii. introducing into said cells a survival plasmid comprising a dominant-negative mutant of said gene of interest, a second reporter gene, a selectable marker, an origin of DNA replication and a nuclear antigen gene essential for replication of the plasmid within said cells, wherein said survival plasmid is spontaneously lost from said cells;

iv. growing said cells in the presence of a selection compound which selects for said selectable marker;

v. selecting cell clones stably expressing said second reporter gene and said dominant-negative mutant of said gene of interest;

vi. adding the drugs destined for screening their ability to impose selective pressure enforcing retention of the spontaneously lost survival plasmid

vii. determining survival plasmid retention in cells, thus identifying a drug having a a synthetic lethal property when in combination with the mutant or normal gene of interest which is overexpressed.

67. (Withdrawn) The method according to Claim 66, wherein said selectable marker is a dominant selectable marker.

68. (Withdrawn) The method according to Claim 66, wherein said cells are human cells.

69. (Withdrawn) The method according to Claim 66, wherein said cells are rodent cells.

70. (Withdrawn) The method according to Claim 66, wherein the products of said first and second reporter genes are fluorescent proteins.

71. (Withdrawn) The method according to Claim 70, wherein the product of said first reporter gene has an excitation and/ or emmission peak which differs from the excitation and/ or emmission peak of the product of said second reporter gene.

72. (Withdrawn) The method according to Claim 66, wherein said human cells are human cancer cells.

73. (Withdrawn) The method according to Claim 72, wherein said gene of interest is specifically incapacitated in said human cancer cells.

74. (Withdrawn) The method of claim 66, wherein said drug is a chemical compound, an antisensedeoxyoligonucleotide, , ribozymes, RNA aptamers, synthetic small interfering RNA (siRNA) and peptide aptamers.

75. (Withdrawn) A method for screening a library comprising a plurality of molecules which have a synthetic lethal property when in combination with a mutant or normal gene of interest which is overexpressed, said method comprising the steps of:

i. transfecting a first reporter gene into mammalian cells having a genome comprising a mutant or normal gene of interest which is overexpressed;

ii. selecting clones stably expressing said first reporter gene;

iii. introducing into said cells a survival plasmid comprising a dominant-negative mutant of said gene of interest, a second reporter gene, a selectable marker, an origin of DNA replication and a nuclear antigen gene essential for replication of the plasmid within said cells, wherein said plasmid is spontaneously lost from said cells;

vi. growing said cells in the presence of a selection compound which selects for said selectable marker;

v. selecting cell clones stably expressing said second reporter gene and said dominant-negative mutant of said gene of interest;

vi. adding the library comprising a plurality of molecules in order to identify those that impose selective pressure enforcing the retention of the spontaneously lost survival plasmid

vii. determining survival plasmid retention in cells, thus identifying at least one molecule within a library having a synthetic lethal property when in combination with the mutant or normal gene of interest which is overexpressed.

76. (Withdrawn) The method according to Claim 75, wherein said selectable marker is a dominant selectable marker.

77. (Withdrawn) The method according to Claim 75, wherein said cells are human cells.

78. (Withdrawn) The method according to Claim 75, wherein said cells are rodent cells.

79. (Withdrawn) The method according to Claim 75, wherein the products of said first and second reporter genes are fluorescent proteins.

80. (Withdrawn) The method according to Claim 79, wherein the product of said first reporter gene has an excitation and/ or emmission peak which differs from the excitation and/ or emmission peak of the product of said second reporter gene.

81. (Withdrawn) The method according to Claim 75, wherein said human cells are human cancer cells.

82. (Withdrawn) The method according to Claim 81, wherein said gene of interest is specifically incapacitated in said human cancer cells.

83. (Withdrawn) The method of claim 75, wherein step vii further comprises the step of FACS sorting in order to enrich or isolat cells which retain the survival plasmid.

84. (Cancelled) ~~A kit for screening a molecule comprising a plurality of molecule types in mammalian cells having a genome, in order to identify a said molecule having a gene-specific lethal property in said cell, comprising: an integration plasmid comprising a first reporter gene; a survival plasmid compatible with a mammalian cell comprising a functional copy of a gene of interest or a dominant negative mutant of a gene of interest, a reporter gene, a dominant selectable marker gene, an origin of DNA replication and a nuclear antigen gene essential for replication of the survival plasmid, said survival plasmid being spontaneously lost from said cell.~~

85. (Cancelled) ~~The kit of claim 61 wherein the molecule is a drug or chemical compounds.~~

86. (Withdrawn) A kit for screening a group of DNA molecules in order to identify among them one or more modulators of gene expression which are synergistically lethal to a mammalian cell together with a gene of interest, comprising: an integration plasmid comprising a first reporter gene; a survival plasmid compatible with a mammalian cell comprising a functional copy of a gene of interest or a dominant-negative mutant of a gene of interest, a reporter gene, a dominant selectable marker gene, an origin of DNA replication and a nuclear antigen gene essential for replication of the survival plasmid, said survival plsmid being spontaneously lost from said cell; and a vector vehicle containing a second dominant selectable marker gene and carrying either a human GSE library or a wild-type cDNA library.

87. (Withdrawn) A survival plasmid compatible with a mammalian cell comprising a functional gene of interest, a reporter gene, a dominant selectable marker gene, an origin of DNA replication and a nuclear antigen essential for replication of the episome, said episome being spontaneously lost from said cell, wherein the product of said reporter gene is a mutant green fluorescent protein (GFP).

88. (Withdrawn) A survival plasmid compatible with a mammalian cell comprising a dominant-negative mutant of a gene of interest, a reporter gene, a dominant selectable marker gene. an origin of DNA replication, and a nuclear antigen gene essential for replication of the episome, said episome being spontaneously lost from said cell, wherein the product of said reporter gene is a mutant green fluorescent protein (GFP).